potential role for both protein-protein and protein-RNA interactions in targeting LC3 to the nucleus and nucleolus.

Voltage-gated Na Channels

2893-Pos Board B323
Resting State of S4 Identified for Each Domain of Nav1.2 using Omega Current Technique
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During gating transitions the voltage sensor S4 slides through the narrow so-called gating pore. The positively charged amino acids of S4, arginine (R) or lysine (K) sense the transmembranal electric field and promote S4 in or out. If one or more long R or K is replaced by the short neutral glutamine (Q), a leak (omega-) pore is created through which a leak current, the omega current will flow when S4 is at the appropriate position. The occupancy of this leaky position can then be electrically monitored. Rat brain sodium channels Nav1.2 were studied at high expression in X. laevis oocytes with two-electrode voltage clamping at strong hyperpolarization to force S4 into the resting state. Mutant channels with single gaps R1Q, R2Q or R3Q as well as with double gaps RRn,n+1QQ were tested for the presence of omega leaks. We found unambiguous omega currents for double gaps in domain DI (RR12QQ), DII (RR12QQ), DIII (RR23QQ) and DIV (RR12QQ), indicating the resting position of S4 in each domain. These findings are in contrast to skeletal muscle sodium channels Nav1.4 where single gap omega leaks are reported for DII and DIII. However, our single gap mutants in Nav1.2 produced only very small leak currents similar to artifacts sometimes also occurring at wild-type channels at very strong hyperpolarizing pulses and at least ten times smaller than those with double gaps. Based on this study, we currently use our double gap channel constructs as a tool to selectively investigate which S4 of the four domains I-IV are immobilized by inactivation.

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Selective Immobilization of S4 in Domain III and IV of Rat Brain Nav1.2 Shown by Omega Currents
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Recently, we have identified the minimally necessary number of mutations of the outer positively charged arginine (R) or lysine (K) to glutamine (Q) in the voltage sensor S4 of each domain, producing an inward omega leak current in the resting state. In all four domains a double gap (RRn,n+1QQ) gave distinct omega currents. In this study we use these double gap channel constructs as a tool to investigate which S4 of the four domains I-IV is immobilized by inactivation. The recovery time constant of sodium current after inactivation was measured with a classical double pulse protocol for a wide range of recovery potentials from −100 to −240 mV. In addition, the onset of the omega current at the same recovery potentials was measured twofold: without and with an inactivating prepulse. We found that the onset of omega current was fast and not affected by inactivation in domain I and II; however, in domain III and IV the onset was fast without prepulse but was slowed after the inactivating prepulse. The return to the resting state seems to be hindered due to immobilization. The time constant of the recovery of omega current matches well the recovery of sodium current over the wide potential range studied. We corroborated our results by using the mutation R4H in S4DIV, which slows the sodium current recovery about twentyfold (Kühn and Greeff, 1999). Adding the mutation R4H in S4DIV to our double gap constructs, we found that the omega current was also slowed by the same factor. This suggests that the same mechanism which keeps the alpha pore closed for ionic current in inactivated channels would also hinder the return of S4III and S4IV to the resting position.

2895-Pos Board B325
Voltage Sensor Domains and Closed-State Inactivation in Sodium Channels
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Sodium channels enter into a state of fast inactivation after opening, or directly from closed states. We examined the roles of the four voltage sensor domains in hNaV1.4 in closed-state fast inactivation using a mutagenesis approach. Charge reversing mutations of outer arginine residues in domains I, III and IV depolarized the steady-state fast inactivation curve and accelerated entry. Similar effects on closed-state fast inactivation were observed for charge-reversing mutations of inner negative charges in domains I and IV, suggesting that electrostatic interaction of these residues limits S4 translocation in response to sub-threshold depolarization. This work was supported by NIH 2P20GM103408 to ISU.

2896-Pos Board B326
Gating Pore Currents are Common Defects of Two Nav1.5 Mutations in Patients with Mixed Arrhythmias and Dilated Cardiomyopathy
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The gating pore current, also called omega current, consists of a cation leak through the typically non-conductive voltage sensor domain (VSD) of voltage gated ion channels (VGIC). While the study of gating pore current refined the knowledge of the structure and the function of VGIC, their implication in cardiac disorders has not been established. Two Nav1.5 mutations (R222Q and R225W) localized in the VSD are associated with complex arrhythmias and dilated cardiomyopathy. Using the patch clamp technique, in-silico mutagenesis and molecular dynamic simulations, we tested the hypothesis that these two mutations may generate gating pore currents potentially accounting for their atypical clinical phenotypes. Our findings suggest that the gating pore current generated by the R222Q and R225W mutations could constitute the yet unrevealed pathological mechanism linking Nav1.5 VSD mutations with cardiac arrhythmias and dilatation of cardiac chambers in humans.

2897-Pos Board B327
Interaction of the Cardiac Sodium Channel Alpha-Subunits Leads to Coupled Gating Properties
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Objective: The cardiac sodium channel has been linked to cardiac arrhythmias. We have shown the existence of dominant-negative mutations in Brugada Syndrome due to interactions between alpha-subunits. Here we investigated the stoichiometry of the interaction and whether the interaction leads to coupled gating properties.
Methods: Single-molecule pull-down experiments and blue native gels have been performed to study the stoichiometry of the interaction, while FRET/TIRF experiments were performed to investigate the interaction at the cell surface. Biophysical properties were studied by patch-clamp analysis in the whole-cell configuration.
Results: Biochemistry results support the dimerization of alpha-subunits. FRET/TIRF experiments showed interacting channels at the plasma membrane. Also, we investigated if the dimerization of the channel leads to biophysical consequences. To do so, we used different mutants leading to specific biophysical. R1860X and R1629Q mutants were used for inactivation and R878C for gating deficiency. When cells expressed WT and R1629Q mutant channels, inactivation properties behaved more closely to the WT contrarily to what would be expected of 2 channels working independently. In addition, when R1629Q was coepxressed with the gating deficient mutant R878C we showed a significant improvement of the R1629Q inactivation properties, even though R878C is not conducting. We then used a truncated channel R1860X and once again the coexpression with R878C significantly improved the inactivation defect, which was not the case with the expression of a C-terminus fragment alone. This, strongly suggest that the presence of the full length channel could rescue the inactivation defect of the delta-Cter channel.
Conclusions: Our data indicate that the alpha-subunits of the cardiac sodium channel present coupled gating properties due to the formation of dimers.

2898-Pos Board B328
Superresolution Microscopy Reveals Sodium Channel Localization within Intercalated Disk Microdomains: Implications for Ephaptic Coupling
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Pore-forming (Nav1.5) and auxiliary (β1; SCN1b) subunits of cardiac sodium channels are enriched at the cardiomyocyte intercalated disk (ID). Mathematical models suggest that this may facilitate conduction via ephaptic mechanisms. We previously demonstrated anisotropic conduction slowing during acute interstitial edema (AIE), possibly due to weakened ephaptic coupling. Here we assessed Nav1.5 and β1 localization to ID microdomains using

electron microscopy (EM) and super-resolution microscopy (gSTED, STORM) and used optical mapping and computer modeling to investigate the implications for ephaptic conduction in the heart. gSTED and STORM revealed Nav1.5 and β1 enrichment within ID regions not containing dense clusters of Cx43 and N-Cadherin. Notably, both were identified within the perinexus, a microdomain surrounding Cx43 gap junctions. Overall, 22% of Nav1.5 was located within perinexal regions while only 2% was within Cx43 clusters. EM revealed closer membrane apposition at perinexal (<10nm) vs. non-perinexal intercalated disk sites (>10nm) under control conditions. AIE increased intermembrane distance at perinexal, but not at non-perinexal sites. Functionally, this correlated with decreased transverse conduction velocity (CV-T; 15.2 ± 0.3 vs. 19.6 ± 0.1cm/s) and increased anisotropic ratio (AR; 3.0 ± 0.2 vs. 2.8 ± 0.1) relative to control, in perfused guinea pig ventricles. Next, we investigated AIE effects on Nav1.5 function in conduction. Nav1.5 blockade (0.5 μM flecainide) by itself decreased CV (18%) without changing AR. However, Nav1.5 inhibition during AIE preferentially decreased CV-T (13.0 ± 0.6cm/s), increased AR (3.3 ± 0.2) and increased spontaneous arrhythmias (7/9 vs. 4/11) compared to AIE alone. Notably, only a computer model including ephaptic coupling and the ID localization of Nav1.5 could recapitulate these results. In summary, sodium channel complexes localized to ID microdomains such as the perinexus may enable ephaptic conduction in the heart. Further, Nav1.5 functional availability and perinexal membrane spacing emerge as novel determinants of anisotropic conduction.

2899-Pos Board B329

Loss of Calmodulin-Mediated Regulation of Na^+ Channel Causes Remodeling of Electrical and Junctional Proteins; and Induces Dilated Cardiomyopathy in IQ/$AA^{+/-}$ Mice

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Sodium channel mutations near the IQ and EFL motifs in the carboxyterminal (CT) domain have been linked to long QT (LQTS) and Brugada syndromes (BrS). IQ-calmodulin (CaM) interaction is important for regulation of cardiac Na channels. The aim of this study was to assess the role of Na^+–Ca^{2+}/CaM signaling via IQ motif of the Na^+ channels in development and maturation of intercalated disc (ID). We studied transgenic mice with alanines knocked into IQ positions in the $Na_v1.5$ CT. The homozygous mice are embryonic lethal and heterozygous mice (IQ/$AA^{+/-}$ mice), develop cardiomyopathy (DCM). We measured the signal and distribution of $Na_v1.5$, syntrophin, Cx43 and ryanodine in 3 and 9 month old IQ/$AA^{+/-}$ mice. Results were compared to those obtained from age matched wild type mice. By immunohistochemistry we show that $Na_v1.5$ protein in 9 month-old IQ/$AA^{+/-}$ mice is significantly reduced at the ID. Syntrophin that traffics Na channels to the membrane, is not altered. Cx43 which is co-located with $Na_v1.5$ at the ID, is significantly reduced. The expression of these proteins were not altered in 3 month-old IQ/$AA^{+/-}$ mice. We also assessed the implication of IQ domain on the localization of Ca^{2+} handling protein such as ryanodine receptor and found that it was significantly altered in 9 month-old IQ/$AA^{+/-}$ mice. The data suggest that enhanced late $I_{Na,L}$ in IQ/$AA^{+/-}$ mice contributes to DCM via remodeling of electrical and junctional proteins and demonstrate a dynamic interplay of Na^+–Ca^{2+}/CaM signaling via IQ motif of the Na^+ channels in ID development and maturation. Our study highlights the importance of Ca^{2+}/CaM-mediated regulation of Na^+ channels in DCM and arrhythmia.

2900-Pos Board B330

Mutation Specific Drug Response and Cardiac Risk in Long QT Type 3

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Long QT type 3 (LQT3) is caused by mutations that cause an increase in the cardiac sodium current during late phases of the cardiac action potential. For a number of LQT3 mutants this is caused by a failure to inactivate fully, and a consequent increase in late sodium current. For several mutations, shifts in voltage dependence of activation and inactivation cause increased sodium channel contribution to more depolarized voltages, an effect referred as increase in window current. The goal of this study was to perform a study in a large number of LQT3 associated mutations and compare the biophysical effect of the mutation to cardiac risk in LQT3 patients and response to treatment. We measured the function of eight common mutations associated with LQT3 with different mechanism underlying channel dysfunction. We compared the functional effects of the channel with the clinical course of these patients and observed that for the two mutations tested with increased sustained current but without increase in current availability (window current), D1784K and D1790G, the patients had significant lower risk of cardiac events, suggesting an increase in current availability may be associated with increased risk for these patients. In addition, we measured mutation specific effects of ranolazine for these mutants. For all mutants tested, ranolazine preferentially blocked late sodium currents. Ranolazine shifts steady-state availability of the inactivation was dependent on the specific mutant tested. Our results suggest that ranolazine may have a mutation dependent effect. Mutations dysfunction may affect drug binding and drug actions in the channel and may alter treatment effectiveness in patients.

2901-Pos Board B331

Rotational Symmetry of Two Pyrethroid Receptor Sites in the Mosquito Sodium Channel

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Pyrethroid insecticides target voltage-gated sodium channels. Emerging mosquito resistance to widely used pyrethroids demands development of new insecticides. Earlier the X-ray structure of the open Kv1.2 channel and mutagenesis data were used to build two homology models of insect sodium channels with pyrethroid receptors PyR1 (O'Reilly et al., 2006) and PyR2 (Du et al., 2013) located, respectively, in the II/III and I/II domain interfaces. The models differ in the number of contributing transmembrane helices, orientation of the bound pyrethroid molecules, and the depth of their penetration in respective domain interfaces. Here we employed our PyR2 model to elaborate an analogous PyR1 model. Computational docking yielded a revised PyR1 model with deltamethrin bound between the linker helix IIS4-S5 and transmembrane helices IIS5, IIS6 and IIIS6 with its dibromoethenyl and diphenylether moieties oriented, respectively, in the intra- and extracellular directions. Comparison of the PyR2 and revised PyR1 models predicted new deltamethrin-channel contacts. Model-driven mutagenesis followed by electrophysiological measurements unveiled two new pyrethroid-sensing residues in PyR1 and four such residues in PyR2. Taken together, the new and previously published data support the following conclusions. (i) PyR1 is formed by helices IIS4-S5, IIS5, IIS6, and IIIS6. PyR2 is formed by helices IS4-S5, IS5, IS6, and IIS6. (ii) Helix IIS6 contains four residues that contribute to PyR1 and four residues that contribute to PyR2. (iii) Seven pairs of pyrethroid-sensing residues are located in analogous positions of domain interfaces I/II and II/III indicating rotational symmetry of the two pyrethroid receptor sites. (iv) Pyrethroids bind to both sites in similar orientations, deeply penetrating in the respective domain interfaces. Our study elaborates the dual pyrethroid-receptor sites model and provides a structural background for rational development of new pyrethroid insecticides. Supported by NIH and NSERC.

2902-Pos Board B332

Nav1.7 Inhibitor, PF-05089771, Inhibits Fast- and Slow-Inactivated Channels with Similar Affinities

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Voltage-gated sodium channel (Nav) inhibitors are used clinically as analgesics and local anesthetics. However, the absence of Nav channel isoform selectivity of current treatment options can result in adverse cardiac and CNS side effects, limiting their therapeutic utility. Human hereditary gain- or loss-of-pain disorders have demonstrated an essential role of Nav1.7 sodium channels in the sensation of pain, thus making this channel an attractive target for new pain therapies. We have identified a novel, human Nav1.7 selective inhibitor (PF-05089771, IC50 = 11 nM) that preferentially interacts with, and stabilizes, inactivated conformation(s) of the channel via an interaction with the voltage-sensor domain (VSD) of Domain 4. The current study demonstrates that PF-05089771 exhibits concentration-dependent slowly developing inhibition (tau = 209 sec and 33 sec, at 100 nM and 1 μM, respectively), and a similarly slow recovery from block upon washout (tau ~7 min). PF-05089771 exhibits minimal use-dependent inhibition until concentrations exceed 10-fold the IC50, which is consistent with the observed slow onset of block and/or a low affinity for resting or fast-inactivated channel conformations. To evaluate this further, we employed whole cell patch clamp protocols to separate channels into predominantly fast- or slow-inactivated Nav populations. Inhibition by PF-05089771 develops with similar rates using protocols that biases for either fast- or slow-inactivated states, suggesting that preference for a particular inactivated state (fast, intermediate or slow) appears less critical than the relative time that the channel is in an inactivated state during